Does restored plant diversity play a role in the reproductive functionality of Banksia populations?

Running head: Reproductive functionality of restored keystone species.

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Abstract

Vegetation structure and plant species diversity of restoration sites are predicted to directly affect pollinator attraction, with potential impacts on gene flow, reproduction, genetic diversity of future generations and ultimately restoration success. We compared *Banksia attenuata* R.Br. (Proteaceae) in a low species diversity restoration site and an adjacent natural remnant. We assessed fecundity genetic diversity in adult plants and their offspring, mating system parameters and pollen dispersal using paternity assignment. Results were compared to an earlier study of reproductive functionality within a high species diversity restoration site that was restored in a similar manner, enabling us to investigate any association between plant species diversity and fecundity. Seed set data indicated no significant differences between restored and adjacent natural sites, however, seed set between restoration sites was significantly different (2.08 ± 0.39 and 6.89 ± 1.12, respectively). The mean number of fruits (follicles) per inflorescence was not significantly different between restoration sites. Genetic diversity of adult plants and their offspring were comparable in all sites. Higher allelic richness and genetic differentiation in one restored site reflected sourcing beyond local provenance. Low correlated paternity indicated high levels of multiple siring of seeds and paternity assignment demonstrated strong genetic connectivity between sites. Reproductive functionality, as measured by fecundity and genetic diversity in the offspring of *B. attenuata*, is resilient to low species diversity within a restored plant community. We consider our results in the context of establishing Seed Production Areas (SPAs) that maximize the quantity and genetic quality of *Banksia* seeds for restoration.

**Key words:** pollinator services; ecological restoration; *Banksia attenuata*; paternity; mating system; connectivity.
Implications for practice

- No association was found between restored community species diversity and reproductive functionality of *Banksia attenuata*, as measured by fecundity, genetic diversity of offspring and genetic connectivity.
- Pollination by highly mobile nectar-feeding birds can provide resilience against the predicted negative impacts of low species diversity and habitat fragmentation on fecundity, genetic diversity and connectivity in restored plant populations.
- Our results suggest that low species diversity/monoculture establishment of Seed Production Areas (SPAs) may not negatively affect the delivery of seed production objectives for outcrossing bird-pollinated plants, although more work is required to understand thresholds of connectivity.
- Restoration sites can be used for single or multiple purposes – such as restoration of biodiversity and SPAs – in either case, they should not be considered in isolation. An understanding of pollinator ecology may be required in respect to SPA placement in the landscape.

Introduction

The composition and spatial structure of plant communities directly affects ecosystem functionality (Naeem et al. 1994). Increasingly, ecological restoration strives to not only re-establish plant communities, but to also restore resilient, functioning ecosystems. The re-establishment of ecological function requires restoration of the dynamic attributes of ecosystems, which includes the interactions among organisms in their environment (SER 2004). Numerous conceptual models have been developed to describe the association between structure and function in an ecological restoration context (Hobbs & Norton 1996; Cortina et al. 2006; Fortuna & Bascompte 2006). These ecological models are used to aid
restoration project planning and are applied to predict the outcomes of restoration (Anand & Desrochers 2004; Cale et al. 2009). However, these models and frameworks are based on the principles that reduced ecosystem structure and species diversity reduces ecosystem functioning (Bartha et al. 2004; Suding & Hobbs 2009). There have been few attempts to test this empirically (Lindenmayer et al. 2008) especially in a restoration context. The practice of ecological restoration, however, provides a powerful experimental setting to do so (Suding & Hobbs 2009).

Species composition and spatial structure of plant communities established during restoration can directly affect the attraction, abundance and behavior of pollinators, influence pollen flow, and therefore mating systems (Wilcock & Neiland 2002; Ghazoul 2005). Density of reproductive plants affects foraging and flight movements of pollinators and pollinator attraction (Wilcock & Neiland 2002; Knight et al. 2005). For species dependent on biotic pollinators within a restored site, a cascade of issues can arise from reduced pollinators. Decreased pollen flow, increased inbreeding, reduced seed set in outcrossed species resulting in fewer seedlings, increased population genetic structure and reduced gene flow may result (Ghazoul 2005; García-Robledo 2010).

Research on the restoration of inter-specific interactions are restored, or how restoration methods can alter pollination systems, is in its infancy (Dixon 2009; García-Robledo 2010; Menz et al. 2011). Several recent studies have begun to understand this research gap through a genetic assessment of contemporary mating patterns and pollen dispersal within and among natural and restored communities addresses these gaps (Ritchie & Krauss 2012; Frick et al. 2014; Mijangos et al. 2015). Frequently, an objective of ecological restoration is to promote connectivity and integrate the restored community with the surrounding landscape, thereby increasing restored ecosystem resilience (Hobbs & Norton 1996; Lundberg & Moberg 2003; McKay et al. 2005). Selection of source material provenance therefore needs careful
consideration, as restoration is most likely to be successful if source material is of high
genetic diversity, suitably adapted to the local restoration site conditions, and free from the
deleterious effects of inbreeding, for avoidance of maladaptation and outbreeding depression
(Charlesworth & Charlesworth 1987; Hufford & Mazer 2003; O'Brien et al. 2007; Hufford et

Here, we utilize a unique experimental opportunity to retrospectively evaluate the
association between initial species diversity and reproductive functionality of a dominant tree
in two 16yo post-sand-mining restoration sites of Banksia woodland. These sites were
established to address the regulator requirements on the mining company for rehabilitation
post-mining, not explicitly for our current objectives. As such, there are limitations in
experimental design. However, optimal replication and experimental control of a study at this
spatial (multiple sites of many hectares involving restoration of hundreds of species) and
temporal (almost two decades) scale is almost impossible. Despite this, we suggest that the
broad nature of our conclusions provide biologically meaningful insight into resilience of
reproductive functionality in this system. Our conclusions also have implications for the
establishment of Seed Production Areas (SPAs) for restoration, an increasingly important
solution for overcoming shortfalls in seed availability for effective restoration, maximizing
seed supply and managing genetic diversity (Nevill et al. in press; Broadhurst et al. 2016).

We evaluated fecundity, genetic diversity, mating system parameters, and genetic
connectivity of Banksia attenuata within a restoration site established with low plant species
diversity. The results are compared with a previously published study on the same species at
a nearby high species diversity restoration that was otherwise practically identical (Ritchie &
Krauss 2012). This high diversity site reinstated over 70% of the 172 species found within 18
ha of adjacent intact Banksia woodland (Petroleum 2011), while the low diversity site
reinstated only 6% of species diversity (Clarke & Langley 2000). In this way, we assessed the
association of initial species diversity at restoration sites with reproductive functionality, genetic diversity and connectivity. Specifically, we:

(i) Compared reproductive output, genetic diversity, mating system parameters and connectivity in *B. attenuata* between a restoration site established with low plant species diversity and an adjacent natural site;

(ii) Contrasted the above measures with those obtained in a similar study for *B. attenuata* in a restoration site established with high plant species diversity and its adjacent natural site (Ritchie & Krauss 2012);

(iii) Assess the implications of our results for ecological restoration, and in particular, for SPAs needed to generate the quantity and genetic quality of seed required for many large-scale restoration activities.

**Methods**

**Study species**

*Banksia attenuata* R.Br. (Proteaceae), is a woody tree or shrub species growing on deep sand, and is dominant in *Banksia* woodlands across the Swan Coastal Plain of Western Australia (George & Gardner 1981). *Banksia* woodlands once extended more than 7300 km², but now less than 50% remain (Tulloch et al. 2015), with only 7% in conservation reserves (Lamont et al. 2007). The species is used extensively in ecological restoration (Rokich 2016), and is an obligate outcrosser (Scott 1980; Ritchie & Krauss 2012) that relies on pollination by nectar-feeding birds (predominantly honeyeaters in the family Meliphagidae), small marsupials, and to a lesser extent bees and wasps (Collins & Rebelo 1987; He et al. 2009). The species has thousands of brilliant yellow sessile florets forming inflorescences, arranged orthogonally around a central woody axis forming flower spikes up to 5 cm wide and up to 25-30cm long (George & Gardner 1981). Anthesis proceeds acropetally over 10 to 20 days and is
asynchronous. The inflorescences are produced over several weeks from spring to late summer, and thus have different stages of development over the flowering season (Wooller & Wooller 2001). Oval follicles develop and mature over 7-8 months with seed development taking over 4 months (Stock et al. 1991). Consequently, it is vital that these plant-pollinator interactions are restored for successful outcrossed seed set as the species is self-incompatible and as few as 0.1% of florets may develop into follicles (Cowling & Lamont 1987).

**Study location and assessment of plant species diversity**

This study was conducted at Jandakot (32 06’28”S, 115 52’01”E), 21 km southeast of the Perth Central Business District (CBD), Western Australia. The restored site (with approximately 120 adult *B. attenuata* trees) is located within a 57 ha post-sand-extraction site (referred to as “Jandakot restored”) leased by Rocla Quarry Products. The adjacent bushland (with approximately 500 adult *B. attenuata* trees) is a naturally occurring 72 ha remnant of *Banksia* woodland (referred to as “Jandakot natural”; Fig. 1). The two sites are in close geographical proximity (circa 150 m). The *B. attenuata* plants in the restored site were 16 years old when this study commenced in 2011. Plants within the adjacent natural site were adult individuals estimated at up to 300 years old (Lamont et al. 2007). Results of the current study at Jandakot were compared to a similar assessment of restoration success (Ritchie & Krauss 2012) within an environmental award winning restoration site owned by Rocla Quarry Products located at Gnangara (31 47’09”S, 115 56’32”E), 40 kmnorth-northeast of the Perth CBD (Fig. 1), restored in 1995. The Gnangara natural site (with approximately 350 trees) and restored site (with approximately 200 trees) are also in close geographical proximity (circa 200 m). The Gnangara and Jandakot areas are both situated on the Bassendean dune system, which is characterized by low - nutrient, acidic, and low water holding siliceous sands (Gozzard & Mouritz 1989). Mining operations at both sites removed
20-30 m of soil from the profile to 3 m above the water table, followed by comparable and parallel reconstruction of substrates for post-mining restoration of the plant community (RPS Consultants, *unpublished report*). At the time of this study, there were no other *Banksia* woodland restoration sites adjacent to natural remnants that contained *B. attenuata* trees in a reproductive stage of development, which restricted our ability to achieve a desired level of replication.

Plant species diversity and vegetation structure was measured as species presence and growth form (according to Clarke & Langley (2000)) within restored and adjacent natural sites at Jandakot and Gnangara. Survey data were collected for the Jandakot restored site using ten 10 x 10 m quadrats in Summer 2010 and the Jandakot Regional Park (as reference for the natural adjacent site) in Spring 2000 by Bush Forever using ten 10 x 10 m quadrats (Clarke & Langley 2000). The Gnangara sites were surveyed in Spring 2012 with 20 x 20 m quadrats, 20 in the natural remnant site and 51 in the restored site (RPS Consultants, *unpublished data*).

Plant species diversity was estimated within restored and natural remnants using Sorenson’s Similarity Index: 

\[
\text{Sorenson’s Similarity Index} = \frac{2c}{S_1 + S_2},
\]

where \(c\) is the number of species in common, \(S_1\) is the number of species at site 1, and \(S_2\) is the number of species at site 2. This index ranges from 0 (no species overlap) to 1 (complete overlap) (Sørensen 1948).

Analyses on growth form data were carried out using PRIMER v6 (Clarke & Warwick 2001). The data were square-root transformed and used to create a Bray-Curtis distance matrix (Legendre & Legendre 1998). Bray-Curtis distances were used to perform an analysis of similarity (ANOSIM) to test for differences in vegetation structure among sites (Clarke & Warwick 2001). A Similarity of Percentages (SIMPER) analysis was used to identify the
percentage similarity between sites and the plant species that were most important in discriminating among sites.

**Assessment of Reproductive Output**

Reproductive output for *B. attenuata* was measured in the restored and natural sites at Jandakot and Gnangara. Ten adult trees were selected randomly per site, recording viable seed production (through visual examination of the seeds) in one year (2010), and new inflorescences produced and the number of follicles per inflorescence over two years (2011, 2012). Number of follicles was used as a reliable indicator of seed production in 2011 and 2012 as each follicle produces up to 2 seed, and we found that mean number of seed per follicle was consistent across sites (mean seed per follicle in 2010 range = 0.61 – 0.87; $F_{[3,39]} = 0.96, P = 0.42$). Variation in reproductive output was assessed using repeated measures of Analyses of Variance (ANOVA) to assess seed production per inflorescence and mean number of follicles produced per inflorescence. When a significant ($P < 0.05$) result was observed site means were compared using Tukey’s post-hoc tests. The correlation between inflorescences produced and annual rainfall was assessed by regression analysis.

**Genetic Sampling and Genotyping**

Four young leaves were collected from each sampled adult tree at Jandakot in 2011 and stored with silica gel crystals until DNA extraction (restored, n = 99; natural, n = 103). The exact location of each sampled tree was determined by Global Positioning System (GPS) to enable construction of a spatial distribution map (Fig. 1). These included the ten selected maternal trees monitored for reproductive output for which a minimum of ten seeds were collected from each cone.
DNA was extracted from 0.8 g silica-dried leaf material using a CTAB (cetyltrimethyl ammonium bromide)-based procedure (Doyle & Doyle 1990; He et al. 2004). DNA was extracted from seeds using the extraction protocol of Jobes et al. (1995). Seed coats were separated from their radicles to avoid contamination of DNA by maternal tissue, then pulverized for 20 seconds in a FastPrep®-24 instrument (MP Biomedicals, Inc., Solon, OH). Microsatellite amplifications were performed for seven polymorphic markers; **BaA3**, **BaA112**, **BaC3**, **Ba5**, **BaB106**, **BaC8**, **BaC112**, previously designed for *B. attenuata* (He et al. 2007). Amplifications were performed with the following PCR conditions: 96°C for 2 minutes (1 cycle), followed by 30 cycles 94°C for 1 min, 52 to 54°C for 1 min (depending on the primer pair; (He et al. 2007), 72°C for 1 min and final extension time of 72°C for 4 minutes. PCR products were separated by capillary electrophoresis and alleles were scored using a Beckman Coulter CEQ 8800 Genetic Analysis System (internal size standard SS400).

Population genetic parameters were estimated for adult and offspring populations using the software program GenAlEx 6.5 (Peakall & Smouse 2012). Genetic parameters estimated included the number of alleles (*N*<sub>a</sub>), effective number of alleles (*N*<sub>e</sub>), number of private alleles (*P*<sub>r</sub>), observed heterozygosity (*H*<sub>o</sub>), expected heterozygosity (*H*<sub>e</sub>), inbreeding coefficient (*F*<sub>IS</sub>). The program FSTAT (Goudet 2001) was used to estimate allelic richness (*A*r) that is corrected for samples of different sizes, and pairwise population differentiation (*F*<sub>ST</sub>) allowing comparison among restored and natural populations. Significant differences between parameter means were noted where 95% confidence intervals did not overlap.

Spatial genetic structure (SGS) within sites was estimated using spatial autocorrelation analysis within GenAlEx 6.5. Distance classes of 10 m (20 in total) were used for direct comparison with results from the Gnangara sites (Ritchie & Krauss 2012). Overall SGS within each site was also assessed using the *S*p statistic to directly quantify the magnitude of SGS among sites (Vekemans & Hardy 2004). This was performed with 10 000 permutations.
using SPAGeDi 1.3 (Hardy & Vekemans 2002). The inverse of $Sp$ was calculated as an indirect estimate of Wright's neighbourhood size, $Nb$ ($Nb = 1/Sp$) for each population.

Mating system parameters (maximum-likelihood single ($t_s$) and multilocus ($t_m$) outcrossing rates, bi-parental inbreeding ($t_{s-t_m}$), correlated paternity ($r_p$) and the effective number of pollen donors per family ($1/r_p$) were estimated using the program MLTR version 3.4 (Smouse et al. 2001; Ritland 2002). 95% confidence intervals were generated for all estimates by bootstrap resampling 100 times.

Likelihood-based paternity analysis was conducted using the program CERVUS 2.0 (Marshall et al. 1998; Kalinowski et al. 2007). The simulation parameters used to assign paternity to the most-likely individuals with a known level of statistical confidence were set at 10,000 cycles of simulation, 204 candidate parents (all sampled reproductive adults from both restored and natural sites), 0.01 as the proportion of loci mistyped (genotyping error rate), and confidence levels of 95% and 80%. The offspring that were assigned to a pollen donor were plotted as a function of inter-tree distance between the known maternal tree and most-likely pollen donor tree.

Differentiation in maternal tree pollen pools ($\Phi_h$) and effective density ($d$) across populations were estimated with TwoGener analysis using GenAlEx 6.5. Overall genetic differentiation between pollen pools ($\Phi_h$) and its variance was calculated following Smouse et al. (2001). The effective number of pollen donors per family ($N_{ep}$) was estimated by $1/(2\Phi_h)$ and the effective pollination neighborhood area ($A_{ep}=N_{ep}/d$ (ha)).

Results

Here we present the results collected from this study at Jandakot (natural and restored) and newly obtained survey (plant diversity and reproduction output) results at Gnangara. Results
from an earlier genetic assessment conducted at Gnangara (Ritchie & Krauss 2012) are included within the tables for direct comparisons and to aid the discussion.

**Site diversity**

Similarity of species diversity between the Jandakot restored and the Jandakot reference site was low ($\beta = 0.09$; SIMPER = 1.3%). Twenty-one plant species were recorded in the Jandakot restored site. Of these, 43% were non-local (i.e. outside their natural distribution). In comparison, the Gnangara restored site and its natural reference site had relatively high similarity ($\beta = 0.62$; SIMPER = 69.2%) and all species were of local origin. Gnangara restored had much higher species diversity than Jandakot restored (Fig. 2), with a total of 184 species; 149 were shared with the reference site and an additional 35 were also recorded. A comparison between reference sites displayed moderate diversity levels ($\beta = 0.36$ and SIMPER = 44.7%). Both restored sites included three dominant overstorey species, *Battarerea attenuata*, *B. menziesii* and *Eucalyptus todtiana*. However, Jandakot restored lacked vegetation complexity within the understory in comparison to the diverse structural components present within both natural sites, and the restored site at Gnangara (Fig. 3, ANOSIM, $R = 0.50$, $P = 0.03$ between all sites; SIMPER = 4.7% between restored sites).

**Reproductive output**

There was no significant difference in the number of viable *B. attenuata* seed produced per inflorescence between the Jandakot restored (2.08 ± 0.39) and its adjacent natural site (2.71 ± 0.30) sites. However, the Jandakot restored site had significantly lower seed production per inflorescence in comparison to the Gnangara restored site (6.89 ± 1.12) (one-way ANOVA $F_{[3,36]} = 8.46$, $P < 0.001$). Comparisons between years showed no significant difference ($F_{[1,72]} = 0.67$, $P = 0.41$) in follicle production per inflorescence between 2011 and 2012,
therefore a two-way ANOVA that pooled years of follicles produced per inflorescence (response variables) was run against site location (Jandakot/Gnangara) and site condition (restored/natural). Mean number of follicles produced per inflorescence (pooled over two years) was significantly lower in the Jandakot restored site (4.27 ± 0.85) only in comparison to the Gnangara natural (7.19 ± 0.68) site (one-way ANOVA $F_{[3,76]} = 2.67, P = 0.005$) (Fig. 4).

There was a significant difference between site location for mean number of follicles produced per inflorescence (two-way ANOVA $F_{[3,76]} = 6.33, P = 0.014$), however this was due to the difference in production between Jandakot restored and Gnangara natural (Table S1 Supporting Information) and was not correlated with annual rainfall ($r^2 = 0.02, P = 0.16$).

**Jandakot genetic diversity and spatial genetic structure**

There was no significant difference in genetic diversity estimates between adult trees and their offspring in the restored and natural sites at Jandakot (Table S2). Mean allelic richness was greatest in the Jandakot restored site (11.05 ± 1.85). There was significant genetic differentiation between restored and natural sites at Jandakot ($F_{ST} = 0.071, P < 0.001$).

Spatial autocorrelation analysis identified significant spatial genetic structure up to an interplant distance of 10 m within the Jandakot natural site ($r = 0.54; P = 0.005$), but no spatial genetic structure was detected within the Jandakot restored site. The kinship coefficient was also low (Jandakot; natural, $Sp = 0.002$, restored, $Sp = 0.001$), with correspondingly large neighborhood size estimates ($Nb$ (natural) = 476 and $Nb$ (restored) = 819).

**Jandakot mating system and pollen dispersal**

Estimates of single and multilocus outcrossing rates were not significantly different from 1.0 in both sites (Table S3). The bi-parental inbreeding estimate was higher in Jandakot natural
than Jandakot restored (Table S3). Estimates of correlated paternity were low in natural and restored sites (10.8 and 10.1%, respectively) and translated into estimates of 9.3 and 9.9 pollen donors per family (Table S3).

The total exclusion probabilities over all seven loci for the first parents were greater (3.752) than the second parents (0.449). Paternity was assigned in 84 out of 199 genotyped seeds (42%), with 80% confidence and 42 (21%) with 95% confidence. For the 84 seeds assigned paternity, 41 were from the Jandakot restored site and 43 seeds were from the Jandakot natural site. Within the restored site, 22 offspring were assigned paternity to a pollen donor within the restored site and 19 were assigned paternity to a pollen donor from the natural site. Within the natural site, 17 were assigned paternity to a pollen donor within the natural site, and 26 were assigned to a pollen donor from the restored site. The average distance of pollen movement from paternity assignments was 171 m (range 4 - 377 m) (Fig. 5).

Statistical differentiation among sampled pollen pools showed that populations of paternal pollen haplotypes sampled from families within the restored site were more differentiated than those sampled within the adjacent natural site (Φsts Jandakot natural = 0.118, Jandakot restored = 0.176; Table S3).

**Discussion**

This study addressed the predicted positive relationship between the species diversity of a restored plant community and its ecosystem functionality (Quijas et al. 2010). Our ecological genetic assessment of reproductive functionality and connectivity in *Banksia attenuata* found no clear association between reproductive fecundity and species diversity despite an order of magnitude difference in initial plant species diversity between two restoration sites. Wide outcrossing, high genetic diversity in offspring and strong connectivity through pollen flow
between adjacent natural and restored sites was found, irrespective of plant species diversity
in the restoration sites. Our results suggest that reproductive functionality, as measured by
fecundity and genetic diversity in the offspring of *B. attenuata*, is resilient to species diversity
within a restored plant community. This is at least in part due to highly mobile, generalist
pollinators (honeyeaters Newland & Wooller 1985), self-incompatibility, wide outcrossing,
lack of SGS (*Sp* < 0.002), and individual longevity resulting in overlapping generations.

Whilst the study design limits our capacity to generalize on direct effects due to
confounding effects such as inter-annual variation, the proximity of the sites to natural
vegetation, and the presence of highly mobile bird pollinators, we suggest that the broad
nature of our conclusions provide a meaningful insight into resilience of reproductive
functionality in restored systems.

Connectivity is a key element of functionality and a central component of ecosystem
resilience (Lundberg & Moberg 2003; Lindenmayer et al. 2008). Connectivity includes both
structural (physical characteristics of a landscape that allow for movement) and functional
(movement of genes, propagules, individuals, or populations) components (Rudnick et al.
2012). Our results demonstrated functional connectivity through pollen dispersal between
adjacent natural and restored sites, with multiple pollen donors in both sites and greater
differentiation of paternal pollen pools within the restored sites. Highly mobile nectarivorous
bird pollinators have also been found to dampen the negative effects of fragmentation on
mating system parameters, such as decreased outcrossing (Byrne et al. 2007; Breed et al.
2013; Lowe et al. 2015). Collectively, these studies suggest that pollination by highly mobile
vertebrate pollinators confers resilience to habitat fragmentation as well as local site species
diversity effects at this scale (< 500m, see Ritchie & Krauss 2012; Frick et al. 2014)

Connectivity is a potentially negative issue if genetic divergence results in outbreeding
depression between mates from distinct provenances (Broadhurst & Young 2007; Hufford et
Our genetic results indicate that although *B. attenuata* propagules sourced for the Jandakot restoration site were genetically diverse, there was significant genetic differentiation between the restored and natural site ($F_{ST} = 0.071$), suggesting provenance of multiple non-local sources. This was in strong contrast to the high species diversity at Gnangara, with very weak genetic differentiation between the natural and restored sites ($F_{ST} = 0.006, p = 0.01$) (Ritchie & Krauss 2012), indicating local provenance sourcing. Experimental studies on *Banksia ilicifolia* (Heliyanto et al. 2006) using within and among-population crosses resulted in fitness advantages from wide outcrossing of plants from different populations. Paschke et al. (2002) concluded that increased available pollen donors led to greater seed production, outweighing any harmful consequences of outbreeding depression on offspring fitness. Consequently, we expect fitness benefits, rather than outbreeding depression, from wide outcrossing in *B. attenuata*, and these restoration sites provide a unique opportunity to test these predictions.

Loss of biodiversity within fragmented systems is predicted to result in the functional simplification of pollinator assemblages (bird, marsupial, insect), thus impacting on the ecosystem services they provide (Garibaldi et al. 2011; Isaac et al. 2014). Reproductive output for *B. attenuata* varied between years at low and high species diversity restoration sites (Fig. 4), however, fecundity was less variable among high species diversity sites. This suggests that low diversity sites may be more sensitive to other factors such as distance between sites, available pollinator density and diversity, annual phenology of co-flowering species and plant breeding strategy (see Dalgleish 1999), which ultimately may impact on the long-term viability of restored plant communities.

Findings from our study have implications for the development, utilization and management of SPAs for restoration. Current and future demand for native seeds far exceeds the volume that can be practically, economically, and ethically sourced from the wild, by up
to 1000 orders of magnitude (Merritt & Dixon 2011). The majority of seed collected for restoration in Australia is sourced from the wild (Broadhurst et al. 2015), with the mining industry consuming 70-80% (Mortlock 1998). SPAs are increasingly seen as a solution to providing material for increasingly ambitious global restoration goals (Nevill et al. in press; Tischew et al. 2011). Seed farming operations to date have focused on monocultures of species with short generation cycles (annual), such as native grasses (Cole & Johnston 2006), and/or species that are wind or self-pollinated (Knapp & Rice 1996), as well as tree species for reforestation/agroforestry (e.g. eucalypts and acacias Ball et al. 1995; Hingston et al. 2004). The low species diversity at the Jandakot restoration site is similar to that found in many current SPAs, where typically monocultures are established (but not always), with limited to no understory. Our results suggest that genetically diverse banksias established in a low diversity / monoculture site has no detrimental effect on delivery of pollinator services and the production of genetically diverse seed, at least where established in a similar landscape context as the study sites assessed here.

However, some SPAs have had poor seed yields, with the lack of appropriate pollinators as the suggested cause (Ball et al. 1995). Studies of eucalypts revealed that the amount of pollen reaching the stigma under open-pollination may be the limiting factor in seed production, and with the addition of bee hives can increase seed yield due to higher outcrossing rates (Ball et al. 1995) or decrease due to evasion (Hingston et al. 2004). Although an introduction of hives into *Acacia* plantations resulted in increased pollination, no increase in seed yield occurred, presumably due to self-incompatibility (Moncur et al. 1995). The lack of understory shrubs in woodland restoration and SPAs may reduce pollinator diversity and services (vertebrate and invertebrate), particularly if the site is isolated from natural populations (see Moncur et al. 1995). Consequently, (re-)establishing bird pollinator
services is of the greatest importance for promoting functionality and seed yield in restoration sites containing species reliant on birds for pollination.

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Supporting Information

Table S1. Two-way Analysis of Variance output for mean follicle production per inflorescence of Banksia attenuata, including all pairwise multiple comparison procedures.

Table S2. Genetic diversity parameters for adults and offspring of Banksia attenuata from natural and restored sites (Gnangara data from Ritchie & Krauss 2012).

Table S3. Mating system parameters for natural and restored sites of Banksia attenuata, estimated using open-pollinated offspring (Gnangara data from Ritchie & Krauss 2012).
Figure Legends

Figure 1. Location of the Gnangara and Jandakot sites in the Perth metropolitan area.

Figure 2. Location of *Banksia attenuata* trees in the Jandakot restored (black circles) and natural (white circles) sites and the maternal trees (grey circles) in each site are indicated. For equivalent map of Gnangara site at 250m scale, see Ritchie & Krauss (2012).

Figure 2. Number of plant species present in restored *Banksia* woodland sites at Jandakot and Gnangara and their adjacent remnant reference sites. ‘Non local’ indicates species that are beyond their natural distribution and ‘Not in reference site’ indicates species that are present in the restoration site but absent from the reference site.

Figure 3. Photographs of vegetation structure at each site; A, Jandakot restored; B, Jandakot natural; C, Gnangara restored; and D, Gnangara natural. Photographs taken by A. Ritchie.

Figure 4. Reproductive output of *Banksia attenuata* in natural and restored sites at Jandakot and Gnangara: (a) mean number of follicles per plant and (b) mean number of follicles per inflorescence. Error bars shown are standard errors.

Figure 5. Distance class distributions of pollen flow inferred from paternity analysis as the distance between each maternal plant and pollen donor for *Banksia attenuata* seeds sourced from natural and restored sites.
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Table S1. Two-way Analysis of Variance output for mean follicle production per inflorescence of *Banksia attenuata*, including all pairwise multiple comparison procedures.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site location</td>
<td>1</td>
<td>75.738</td>
<td>75.738</td>
<td>6.337</td>
<td>0.014</td>
</tr>
<tr>
<td>Site condition</td>
<td>1</td>
<td>19.037</td>
<td>19.037</td>
<td>1.593</td>
<td>0.211</td>
</tr>
<tr>
<td>Site location x Site condition</td>
<td>1</td>
<td>1.039</td>
<td>1.039</td>
<td>0.0869</td>
<td>0.769</td>
</tr>
<tr>
<td>Residual</td>
<td>76</td>
<td>908.264</td>
<td>11.951</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>1004.078</td>
<td>12.710</td>
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<td></td>
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</table>

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: Site Location

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>p</th>
<th>q</th>
<th>P</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gnangara vs. Jandakot</td>
<td>1.946</td>
<td>2</td>
<td>3.560</td>
<td>0.014</td>
<td>Yes</td>
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</table>

Comparisons for factor: Site Condition

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>p</th>
<th>q</th>
<th>P</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural vs. Restored</td>
<td>0.976</td>
<td>2</td>
<td>1.785</td>
<td>0.211</td>
<td>No</td>
</tr>
</tbody>
</table>

Comparisons for factor: Site Condition within J

<table>
<thead>
<tr>
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<th>Diff of Means</th>
<th>p</th>
<th>q</th>
<th>P</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural vs. Restored</td>
<td>0.748</td>
<td>2</td>
<td>0.967</td>
<td>0.496</td>
<td>No</td>
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</tbody>
</table>

Comparisons for factor: Site Condition within G

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>p</th>
<th>q</th>
<th>P</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural vs. Restored</td>
<td>1.204</td>
<td>2</td>
<td>1.557</td>
<td>0.275</td>
<td>No</td>
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</table>

Comparisons for factor: Site Location within Restored

<table>
<thead>
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<th>Comparison</th>
<th>Diff of Means</th>
<th>p</th>
<th>q</th>
<th>P</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gnangara vs. Jandakot</td>
<td>1.718</td>
<td>2</td>
<td>2.223</td>
<td>0.120</td>
<td>No</td>
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</tbody>
</table>

Comparisons for factor: Site Location within Natural

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
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<th>q</th>
<th>P</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gnangara vs. Jandakot</td>
<td>2.174</td>
<td>2</td>
<td>2.812</td>
<td>0.050</td>
<td>No</td>
</tr>
</tbody>
</table>
### Table S2

Genetic diversity parameters for adults and offspring of *Banksia attenuata* from natural and restored sites (Gnangara data from Ritchie & Krauss 2012). Values in parentheses are standard errors.

<table>
<thead>
<tr>
<th>Population</th>
<th>Genetic diversity of adults</th>
<th>Genetic diversity of offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>site</td>
<td>n</td>
</tr>
<tr>
<td>Jandakot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>103</td>
<td>8.57</td>
</tr>
<tr>
<td></td>
<td>(6.92-10.22)</td>
<td>(2.50-4.19)</td>
</tr>
<tr>
<td>Restored</td>
<td>99</td>
<td>11.43</td>
</tr>
<tr>
<td></td>
<td>(6.84-16.01)</td>
<td>(2.56-5.19)</td>
</tr>
<tr>
<td>Gnangara</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>94</td>
<td>7.86</td>
</tr>
<tr>
<td></td>
<td>(6.23-9.48)</td>
<td>(1.86-4.72)</td>
</tr>
<tr>
<td>Restored</td>
<td>106</td>
<td>11.00</td>
</tr>
<tr>
<td></td>
<td>(7.42-14.58)</td>
<td>(1.86-5.21)</td>
</tr>
</tbody>
</table>

Mean values (95% confidence intervals in parentheses) are reported. Na, average number of alleles per locus; Ne, average number of effective alleles; Ho, average observed heterozygosity; He, average expected heterozygosity adjusted for sample size; FIS, fixation indices; Pr, number of private alleles; Ar, mean allelic richness adjusted for sample size.
Table S3 Mating system parameters for natural and restored sites of *Banksia attenuata*, estimated using open-pollinated offspring (Gnangara data from Ritchie & Krauss 2012).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Jandakot</th>
<th>Gnangara</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n_{families} = 10$</td>
<td>$n_{families} = 5$</td>
</tr>
<tr>
<td>Reproductive tree density (per ha)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>33.6</td>
<td>50.0</td>
</tr>
<tr>
<td>Restored</td>
<td>35.4</td>
<td>145</td>
</tr>
<tr>
<td><strong>MLTR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multilocus outcrossing rate ($t_m$)</td>
<td>0.985</td>
<td>1.200</td>
</tr>
<tr>
<td></td>
<td>(0.926-1.044)</td>
<td>(1.198-1.202)</td>
</tr>
<tr>
<td>Single locus outcrossing rate ($t_s$)</td>
<td>0.822</td>
<td>1.055</td>
</tr>
<tr>
<td></td>
<td>(0.769-0.875)</td>
<td>(1.056-1.134)</td>
</tr>
<tr>
<td>Biparental inbreeding ($t_m - t_s$)</td>
<td>0.162</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>(0.092-0.232)</td>
<td>(-0.132-0.032)</td>
</tr>
<tr>
<td>Correlated paternity ($r_p$)</td>
<td>0.108</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>(0.038-0.178)</td>
<td>(-0.143-0.291)</td>
</tr>
<tr>
<td>Effective number of pollen donors ($1/ N_{ep}$)</td>
<td>9.3</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>9.9</td>
<td>13.3</td>
</tr>
<tr>
<td><strong>TwoGener</strong></td>
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<td></td>
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<tr>
<td>Differentiation in pollen gene pool among families ($\Phi_b$)</td>
<td>0.118</td>
<td>0.136</td>
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<tr>
<td>Number of pollen donors ($N_{ep}$)</td>
<td>4.2</td>
<td>3.7</td>
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<tr>
<td>Effective pollination neighbourhood area ($A_{ep}$)</td>
<td>0.125</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>0.079</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Mean values (95% confidence intervals) are reported. $t_m$, multilocus outcrossing rate; $t_s$, single locus outcrossing rate; $t_m - t_s$, bi-parental inbreeding rate; $r_p$, correlated paternity; and $1/ N_{ep}$, the inverse of correlated paternity as a measure of the effective number of pollen donors, were estimated using MLTR 3.4 (Ritland 2002). $\Phi_b$, paternity differentiation between families, was estimated based on AMOVA using TwoGener in GenAlEx 6.5 (Smouse *et al.* 2001; Peakall and Smouse, 2012).